NOTE ON THE PIGMENT OF ARBACIA EGG-SECRETION.

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I.

If the unfertilized eggs of *Arbacia punctulata* are allowed to secrete into a small volume of sea-water, the latter, in the course of five or ten minutes, takes on an amber color. The density of the pigment varies with the concentration of the eggs as well as with the time during which they secrete. After an hour, the sea-water is apt to become reddish-brown.

The later discoloration is due, at least in part, to the elimination of echinochrome—a substance which these eggs contain in considerable quantity. Whether the pigment given off during the earlier moments of secretion is also echinochrome remains uncertain since the diagnostic reactions recommended by MacMunn ('85) are not convincing at the dilutions at which it is necessary to work. In exudate of more than "standard" strength I have been unable to see that HCl produces a red tint or that NaOH intensifies the yellow. With precipitated pigment both acid and alkali seem to intensify the yellow color to an extent barely perceptible.

II.

One cannot assume, offhand, that the pigment is either important or negligible in fertilization. In my study of egg-exudate, therefore, I first attempted to decolorize the secretion in order that the lipolysin and agglutinin subsequently recovered by other methods might be either free or relatively free from pigment. For this purpose charcoal cannot be used since it has the serious disadvantage of removing the whole, practically, of the organic reaction system. Moreover, the pigment, if wanted for separate study, cannot be recovered readily from the charcoal.

To isolate the pigment, it is much better to use chloroform as a decolorant. The method is very simple. To a given volume of

fresh exudate, one adds, roughly, half a volume of chloroform and shakes vigorously for fifteen minutes. In this time an emulsion is formed in which the individual globules are remarkably stable. The system, indeed, is a jelly, white in appearance and surprisingly voluminous. In fact, both its volume and stability at first misled me into thinking that I had found a method for precipitating, if not all, at least the greater part of the organic solutes present.

III.

On standing, the jelly separates from both the unemulsified chloroform and the remainder of the exudate.

The degree to which the latter is decolorized varies, among other things, with its age. If the exudate is perfectly fresh, the pigment is removed almost if not quite completely; if the secretion is 36 or more hours old, decolorization is more difficult and the chloroform jelly less stable.

Microscopic examination of the jelly reveals on the surface of each chloroform globule a delicate skin, translucent, with pearly sheen, continuous, yet also with suggestions of extremely fine fibrils. As the chloroform evaporates, this skin wrinkles until finally there remains an empty bag.

Since the original exudate contained sea-salts, I washed the jelly on a filter or shook it for half an hour in several changes, first of fresh, and later of distilled, water. Under this treatment the globules of chloroform break up into still smaller spheres greatly increasing the stability of the system. The jelly can be freed from sea-salts entirely and in this state has been kept for weeks in stoppered bottles.

IV.

The material in the walls of the globules can be recovered simply by permitting the chlorofrom to evaporate. Slight heat naturally facilitates the process. The jelly, also, may be broken down instantaneously by means of 95 per cent. alcohol. The vesicles are permeable for the alchohol and this, itself, is soluble in chloroform. Since the material held in the walls of the globules is insoluble in both alcohol and chloroform, precipitation is inevitable.

Macroscopically, this precipitate appears to be coarsely flocculent. Its color is yellowish-brown. Under the microscope, granules aside, one sees chiefly fibers. These, when dried on a filter, yield thin felt-like sheets which cannot be readily dissolved in either sea-water or fresh. The material is only slightly soluble in acids and alkalis.

V.

The solubilities of the precipitated pigment are such that it is very difficult to test the importance of this material in fertilization. So far, nothing that would merit particular attention has come to light and the conclusion that properties highly significant in fertilization are absent is reinforced by the eggs of the starfish, the sand-dollar, the oyster, *Nereis* and *Fundulus*, none of which, apparently, secrete anything that corresponds at all closely with the *Arbacia* pigment. However, there is one suggestive fact: after removal of the pigment, the *Arbacia* exudate, physically, is a less stable system than before. From unmodified exudate nothing free from sea-salts can be precipitated with 95 per cent. alcohol; with the pigment removed, 95 per cent. alcohol, insufficient to precipitate the sea-salts, throws down the sperm-agglutinating material. It appears therefore as though the pigment in some way stabilized the exudate.

LITERATURE.

MacMunn, C. A.

'85 On the Chromatology of the Blood of Some Invertebrates. Quart. Journ. Mic. Sci., Vol. 25.

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